iDamage: a method to integrate modified DNA into the yeast genome

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Abstract

Genome of all living cell can be altered in several manners: DNA lesions such as abasic sites, DNA adducts, DNA crosslinks (intra- or inter-strand), DNA-protein crosslinks, presence of ribonucleotides… These damages, if not repaired will interfere with DNA replication, altering its fidelity, or blocking it.

In order to study in details the mechanisms of replication of such DNA alterations, we have developed a method by which we insert/integrate a modified DNA into a specific site of the yeast genome. This is achieved by the site-specific integration of a modified plasmid at a chosen locus of the yeast genome, through the use of the Cre/lox recombination system.

In the present work, we have used our method to insert two different single UV lesions into the yeast genome, and studied how the balance between error-free and error-prone lesion bypass is regulated. We show the involvement and the interplay of different specialized DNA polymerases in the bypass of the lesions by translesion synthesis (TLS). More interestingly, we show that the inhibition of homologous recombination, either directly (by the inactivation of *rad51* recombinase) or through its control by preventing the poly-ubiquitination of PCNA (*ubc13* mutant) leads to a strong increase in the use of TLS. Such regulatory aspects of the DNA damage tolerance could not have been observed with previous methods using plasmid or randomly distributed lesion and shows the advantage of our new method.

The very robust and precise integration of any modified DNA at any chosen locus of the yeast genome that we describe here is a powerful tool that will allow exploration of many biological processes related to replication and repair of modified DNA.

Introduction

Genomes of all living organisms are constantly threatened by endogenous and exogenous agents that modify the chemical integrity of DNA and challenge its informational content. Several DNA modifications can alter the genome of every living cells in several manners. Such alteration can lead to replication defects (replication blocks or delays, fork collapse…) or alter replication fidelity. Cells possess several strategies to counteract such replication impediments: DNA repair strategies that remove the obstacle before replication occurs, and DNA Damage Tolerance (DDT) that allows to replicate the genome despite the presence of residual DNA damages. There are two distinct DNA Damage Tolerance mechanisms: i) Translesion Synthesis (TLS), employing specialized DNA polymerases able to insert a nucleotide directly opposite the lesion. This pathway is potentially mutagenic due to the miscoding nature of most damaged nucleotides and to the low fidelity of the TLS polymerases. ii) Damage Avoidance (DA, also named strand switch, copy choice or homology directed gap repair) where the cells use the information of the sister chromatid to circumvent the lesion, insuring survival in an error-free manner (Kuzminov 1999).

The balance between error-free and error-prone mechanisms is important since it defines the level of mutagenesis during lesion bypass. Decades of research have yielded significant advances in our knowledge of genetic pathways and factors contributing to different DDT modes. However, our understanding of the precise molecular mechanisms regulating this important process is far from being complete, as the lack of high-resolution methods allowing to monitor both TLS and DA in a chromosomal context of a living cell presented a major limitation in the study of DNA damage tolerance.

Current *in vivo* approaches to study lesion bypass rely mostly on randomly distributed lesions generated by treating the cells with DNA damaging agents. Such assays allow to monitor toxicity and mutagenesis but they are blind to error-free events and cannot evidence neither error-free TLS, nor DA events. Assays based on plasmids containing a single lesion have been used to overcome these hurdles. However, the use of these plasmids is limited by the relatively small size of the vectors: the functional uncoupling of the replication of both strands after one polymerase has encountered a lesion (Pagès & Fuchs 2003) allows to monitor TLS but not DA (Pagès et al. 2012). Hence, we have a variety of tools available that have been very useful in unravelling the process of TLS, but most essential questions related to DA processes remain unanswered today. When it comes to recombinational repair, most attention has been focused on double-strand break repair. Indeed, the quite recent engineering of HO and I-SceI endonucleases, which allow to introduce a unique double-stranded DNA break at a defined locus in the genome, has allowed a great progress in the field of double-strand break repair. However, the processing of single strand gap repair by homologous recombination (DA pathways) remains largely understudied.

In the present article, we describe a method that allows to insert a single DNA damage (or any modified nucleotide) at a chosen locus of the yeast genome. This method involves the site-specific integration of a vector containing a single DNA modification and a strand marker

that allows to distinguish the damaged from the non-damaged strand upon lesion bypass. The vector is integrated into the genome using site-specific recombination, and a simple colorimetric assay to monitor TLS and DA events.

We have used our method to insert two different UV lesions into the genome of the yeast *Saccharomyces cerevisiae*. We confirm the involvement of several specialized DNA polymerases that has previously been observed using randomly distributed lesions and plasmid assays. In addition, we show that impairing the DA pathway either at the control level (*ubc13*) or at the effector level (*rad51*), leads to an increase in the use of both error-free and mutagenic TLS. Such interplay between TLS and DA can only be evidenced on the chromosomal DNA and at the level of a single lesion. It shows the advantage or our method over currents approaches relying on plasmid-based assays or lesion randomly distributed lesions over the genome.

Results

1. Site-specific integration into the yeast genome

In order to overcome the limitations previously described, we developed a unique assay that allows to follow the fate of a single replication-blocking lesion in the yeast genome. This technique is based on a non-replicative plasmid containing a single lesion, which is stably integrated into one of the chromosomes using site-specific recombination, as previously described for *E. coli* (Pagès et al. 2012; Pagès & Fuchs 2018) (Fig.1). After testing several integration strategies (see supplementary information), we chose a modified version of the Cre/lox system involving left element/right element (LE/RE) lox site mutants. Recombination between LE (lox61) and RE (lox71) lox mutants produces a wild-type loxP site as well as a LE+RE double mutant lox site that is not recognized by Cre (Araki et al. 2010), thus preventing excision of the plasmid once integrated into the chromosome. In addition, if several plasmids enter the cell, once one of them is integrated, the remaining ones cannot be exchanged on the chromosome. Additionally, we placed the Cre recombinase under the control of the doxycycline repressible promoter (Tet-off) so it can be turned off after integration has occurred.

Following ectopic expression of Cre recombinase (pKM34), the plasmid carrying a lesion is introduced by electroporation into a recipient *S. cerevisiae* strain containing a chromosomal integration cassette. The plasmid contains a selectable marker (LEU2), and a single lesion located within the 5′-end of the *lacZ* gene fused to a lox71 site. The chromosomal integration site contains the 3′-end of *lacZ* fused to lox66 site, so that following the precise integration a full-length functional ß-galactosidase gene (*lacZ*) is restored. Integrants are selected on SC-Leu media.

We placed the integration site close to an early replication origin (ARS306 or ARS606) in two different orientations in order to introduce the lesion either on the leading or the lagging strand (Supplementary Fig.S1). The non-damaged opposite strand contains a +2 frameshift inactivating the *lacZ* gene, serving as a genetic marker to allow strand discrimination. After electroporation of the vector, cells are plated on selective indicator plates (SD-LEU, X-gal) before the first cell division occurs. The lesion is placed in such sequence context, that all in-frame TLS events, both error-free and mutagenic, result in a functional *lacZ* gene (blue colony sectors), while DA events result in inactivated *lacZ* gene (white colony sectors). PCR analysis and sequencing confirmed that all colonies obtained on selective plates result from precise integration of the vector into the chromosomal integration site. No colonies were observed after transformation of a strain not expressing Cre recombinase or without chromosomal integration site.

2. Bypass of UV lesions by Translesion synthesis

To validate our system, we constructed 3 integration vectors, containing no lesion, a TT-CPD lesion (cyclobutane pyrimidine dimer) and a TT(6-4) lesion (thymine-thymine pyrimidine(6-4)pyrimidone photoproduct). In order to focus on lesion tolerance mechanisms, we inactivated the repair mechanisms in our parental strain (namely nucleotide excision repair: *rad14*, and photolyase: *phr1*), as well as the mismatch repair system (*msh2)*, to avoid the repair of the strand marker.

Tolerance events are calculated as the ratio of colonies resulting from the integration of damaged vector versus the lesion-free one. Plasmid pRS413 (containing HIS3 marker) (Sikorski & Hieter 1989) was co-transformed and used as an internal control to normalize transformation efficiency between damaged and non-damaged vectors.

After integration of the constructs containing a single CPD or TT(6-4) lesion, no reduction of survival was observed compared to the lesion-free construct (Fig.2). Integration of the heteroduplex containing a single CPD lesion leads to 55% of sectored blue colonies representing TLS events. For TT(6-4) lesion, 3% of TLS events were observed. Those results are in agreement with a previous report by Gibbs et al. (Gibbs et al. 2005), where the authors used gapped-circular vectors containing a single lesion within a short single-stranded region.

In the absence of Pol η (*rad30*), TLS at CPD lesion is strongly reduced to ~18%. The remaining TLS in absence of Pol η is dependent on *REV1* and *REV3* as inactivation of either of these genes in combination with *rad30* leads to an almost complete suppression of TLS events. Despite the drop of 63% in the rate of TLS at the CPD in the absence of Pol η, we observe no loss of survival. This is consistent with the study by Abdulovic and Jinks-Robertson (Abdulovic & Jinks-Robertson 2006) where the authors demonstrated that *rad30* strain is not sensitive to low UV doses. We can therefore conclude that in the presence of low levels of DNA damage homologous recombination-dependent mechanism (DA) can fully compensate for the absence of specialized polymerase.

In the presence of Pol η, inactivation of *REV1* or *REV3* leads to a milder reduction of TLS at a CPD lesion. Both genes are epistatic as the inactivation of both *rev3* and *rev1* leads to the same decrease of TLS. It is interesting to note that Pol η-mediated TLS and Rev1-Rev3-mediated TLS are independent from each other and seem compartmentalized: the drop in TLS in the absence of Pol η cannot be compensated by Rev1-Rev3 TLS and vice versa. Inactivation of all TLS polymerases leads as expected to a complete suppression of TLS events.

RAD30 inactivation has no effect on TLS at TT(6-4) lesions. However, *REV1* or *REV3* (or both) inactivation leads to a complete suppression of TLS at this lesion, showing again the epistasis of both genes in the bypass of this lesion.

Molecular analysis of colonies obtained after lesion integration (Supplementary Table S2) shows that insertion opposite the CPD lesion is 100% error-free in the presence of the 3 specialized polymerases. However, the elongation step is mutagenic in 18% of the cases. The insertion step becomes mutagenic (5%) in the absence of Pol η. Overall mutagenesis (both at the insertion and elongation steps) is almost completely abolished in the absence of *rev3* and *rev1*. The bypass of the TT(6-4) lesion is mutagenic in 30% of the case, mostly due to misincorporation at the insertion step.

These results confirm the genetic data of lesion bypass by TLS polymerases that was previously obtained with replicative or gapped plasmids (Gibbs et al. 2005; Pagès, Johnson, et al. 2008). It confirms that replicative plasmids or gapped plasmids containing a single lesion constitute a good model to study TLS. It also proves that our method by which we introduce a single lesion in the genome is suitable to study TLS.

3. PCNA ubiquitination is an absolute requirement for TLS

It is known that the balance between TLS and DA is regulated by PCNA ubiquitination that occurs in response to DNA damage. PCNA mono-ubiquitination (at Lysine 164) is mediated by Rad6/Rad18 and favors TLS. PCNA poly-ubiquitination depends on Mms2-Ubc13 ubiquitin-conjugating complex and the ubiquitine-ligase activity of Rad5, and is important for DA. We introduced our two UV lesions in strains were PCNA cannot be ubiquitinated, either by the inactivation of *RAD18*, or by the mutation of Lysine 164 of PCNA (*pol30*-K164R) (Fig.3). In both situations, in the absence of PCNA ubiquitination, the TLS level at CPD and TT(6-4) lesions is almost completely abolished. It is interesting to note that in the absence of any DNA-damaging treatment, the presence of a single replication-blocking lesion is sufficient to generate the signal required to trigger Rad6-Rad18-mediated PCNA ubiquitination which is clearly necessary to promote TLS.

4. Lack of PCNA poly-ubiquitination favors TLS

We then looked at how PCNA poly-ubiquitination could affect the ratio DA/TLS (Fig.3). In the absence of PCNA poly-ubiquitination (*ubc13* strain), we observed no significant effect on the bypass of CPD lesion. On the other hand, we observed a more than 10 fold increase in the

Pol ζ-mediated TLS at the TT(6-4) lesion, reaching more than 40%. In the absence of PCNA poly-ubiquitination, DA is reduced and is compensated by an increase in TLS. Such phenomenon has previously been observed in *E. coli* where we showed that a defect in homologous recombination led to increased TLS (Naiman et al. 2016). This observation that a decrease in the DA pathway leads to an increase in TLS is only possible with our method. Previously used approaches based on randomly distributed lesion (e.g. UV irradiation) can reveal an increase in mutagenesis, but are blind to error-free processes (including DA and error-free TLS). It has previously been reported that *ubc13* inactivation led to a ~2-fold increase in UV-induced mutagenesis (Halas et al. 2011), reflecting the low fraction of mutagenic TLS events. We report here a >10 fold increase in the use of Pol ζ-mediated TLS in the *ubc13* strain, our system allowing to monitor both mutagenic and error-free TLS events. Plasmid-based assays that allow to monitor error-free TLS have been used, but they are inappropriate substrates to monitor DA: due to their limited size, the full unwinding of the two DNA strands prevents homologous recombination with the sister chromatid as previously evidenced in *E. coli* (Pagès et al. 2012). Indeed, the inactivation of *ubc13* doesn't lead to an increase of TLS at a single lesion bypassed on a plasmid system either in *S. cerevisiae* or *S. pombe* (Pagès, Bresson, et al. 2008; Coulon et al. 2010).

To confirm that the increase in TLS was due to a decrease in DA, we inactivated Rad51 to prevent homologous recombination that is the main actor of DA. In the *rad51* strain, we observe the same 10-fold increase in TLS rate at the TT(6-4) lesion.

Only the monitoring of a single DNA lesion inserted in the genomic DNA is able to reveal the interplay between DA and TLS.

Discussion

Decades of studies of DNA damage tolerance have yielded significant advances in our knowledge. It is well established that tolerance of DNA lesions is shared between error-prone TLS and error-free DA, and that the former is controlled by PCNA mono-ubiquitination while the latter by PCNA poly-ubiquitination. However, several questions remain regarding how is controlled the balance between these two pathways.

Over the years, many assays have been developed to study TLS and DA. However, their main limitation is that they do not allow to monitor both TLS and DA at the same time. Genome-wide assays involving treatment with DNA damaging agents allows to monitor toxicity and mutagenesis, but are blind to error-free events. The introduction of single lesions onto replicative plasmids have been successfully used to monitor error-free and error-prone TLS (Gibbs et al. 2005; Gibbs & Lawrence 1995; Bresson & Fuchs 2002; Pagès, Johnson, et al. 2008). However, plasmid-based assays are not suited for the analysis of DA events, as during plasmid replication, when a lesion is encountered, replication fork uncoupling leads to full separation of the daughter strands in plasmids, while DA events require close proximity of the two sister chromatids (Pagès et al. 2012).

To overcome the limitations of these approaches, we designed an assay to follow the fate of a single replication-blocking lesion introduced in the genome of a living cell. Our group as previously developed such assay in *Escherichia coli* (Pagès et al. 2012; Pagès & Fuchs 2018), and showed that indeed, such approach allows to monitor both TLS and DA events, and the interplay between these two tolerance mechanisms. It appeared necessary to develop a similar approach in eukaryotic cells in order to explore DNA damage tolerance in this kingdom of life. We chose to use the yeast *Saccharomyces cerevisiae* which provides an invaluable model due to the ease of genetic manipulation and high homology with several human genes. Furthermore, recent progress in construction of yeast strains with humanized genes and pathways opens up many possibilities for the study of human genes and processes in a simpler organismal context (Laurent et al. 2016).

In the present paper, we describe a method to insert a single lesion into the genome of a yeast cell, and to monitor the fate of this lesion in term of lesion tolerance. We introduced two UV lesion (TT(6-4) and CPD) into the genome of *S. cerevisiae*. Our system proved to be suitable to monitor TLS and DA. Indeed, we showed the involvement of the 3 yeast specialized DNA polymerases Pol η, Pol ζ and Rev1 in the bypass of the CPD and TT(6-4) lesions as was previously shown *in vitro* (Johnson et al. 1999) and *in vivo* using gapped plasmids (Gibbs et al. 2005). Several factors have been proposed to regulate the interplay between TLS and DA, among them the nature of the lesion and the post-translational modification of PCNA. We showed that the proportion of TLS vs DA is dependent on the lesion: while TLS represents ~50% of the tolerance pathways for CPD, it represents only ~4% for TT(6-4). For both lesions, no toxicity is observed and DA complement TLS pathway in order to recover 100% of survival (as compared to the integration of the lesion-free control vector). We show that PCNA ubiquitination is an absolute requirement for TLS since no TLS was observed in *rad18* strain

nor in the pol30-K164R, both strains abolishing PCNA ubiquitination. It is interesting to note that in the absence of any DNA-damaging treatment, the presence of a single replication-blocking lesion is sufficient to generate the signal required to trigger Rad6-Rad18-mediated PCNA ubiquitination. On the other hand, DA is still possible in the absence of PCNA ubiquitination since a great proportion of white colonies are still observed both in the rad18 and the pol30-K164R mutants.

Even more interestingly, we show that a defect in the DA pathway leads to an increase in TLS. Indeed, at the TT(6-4) lesion, when the DA pathway is affected either by the inactivation of *ubc13* or by *rad51*, it is compensated by a 10 fold increase in TLS. As we did not observe a similar increase in TLS at the CPD lesion, we hypothesize that the competition between DA and TLS occurs behind the fork, during a gap filling reaction. The majority of CPD lesion is efficiently bypassed by Pol η at the fork. Only the small fraction that is bypass by Pol ζ behind the fork, is in competition with *UBC13*-dependant DA mechanisms for the gap-filling reaction. This hypothesis will need to be confirmed by the use of other strongly-blocking lesions that are bypassed by Pol ζ. In the *ubc13* or *rad51* strains, some DA still persists as we still observe a significant number of white colonies. By adding markers on the damaged and non-damaged strand, we will be able to explore more in details these DA events as previously achieved in E. coli (Laureti et al. 2015).

The increase of TLS in response to inhibition of DA evidenced here could not be observed before in yeast since no assay was able to simultaneously monitor TLS and DA, and therefore the interplay between these two mechanisms. The method described here has the potential to unveil several new aspects of the DNA damage tolerance.

Unlike previous approaches, our system is able to simultaneously monitor TLS and DA, and therefore to explore the balance between these two pathways in a way that was not possible before. DDT pathway choice likely depends on multiple parameters. Many factors may affect this choice including lesion type, sequence context, location in the genome, chromatin state, cell cycle stage, and transcriptional activity. The versatility of our assay permits to explore the impact of those factors. Our assay allows to insert any type of DNA lesion or modification at any desire location in the yeast genome. By placing the damage in centromeric or telomeric regions, highly/poorly transcribed regions, hetero/eu-chromatin regions, near fragile sites etc…, it will be possible to determine how these positions affect the balance between error-free and mutagenic lesion bypass. In addition, the integration site can be placed in two orientations relative to the closest replication origin, allowing to place the lesion on the leading or lagging strand. Similarly, we can choose to insert the lesion on the transcribed, or on the non-transcribed strand of any gene.

This method opens the way to exploration of lesion bypass and single-strand gap repair in the same manner engineering nucleases such as HO or I-SceI has allowed to boost the exploration of double strand breaks repair.

This method is not limited to DNA Damage tolerance, but can also be used to explore several mechanisms related to DNA maintenance, such as repair of DNA adducts, repair of DNA crosslinks, bypass of ribonucleotides inserted into the DNA, effect of DNA-protein

crosslinks, etc… The possibility to integrate vectors carrying any type of damage or chemical group broadens the applicability of our approach beyond the field of DNA damage repair and tolerance.

Materials and Methods

Plasmids

pKM34 expresses the cre integrase under control of a tetracycline-repressible TetO-CYC1 promoter and carries TRP1 selectable marker. The vector is derived from pCM185 (Garí et al. 1997) by cloning cre recombinase from pSH68 (Gueldener et al. 2002) into the HpaI-PstI restriction sites.

pLL43 and pKM71 are derived from pUC18 plasmid and contain: the ampicillin resistance gene and pMB1 bacterial replication origin, yeast LEU2 marker from pSH68, and the 5' end of *lacZ* gene cloned from *Escherichia coli* MG1655 strain in fusion with the lox71 site under control of pTEF promoter from pYM-N18 (Janke et al. 2004).

pRS413 (Sikorski & Hieter 1989) plasmid, carrying HIS3 selectable marker, serves as an internal control for transformation efficiency.

Construction of vectors carrying a single lesion

Duplex plasmids carrying a single lesion were constructed following the gap-duplex method previously described (Koehl et al. 1989). An oligonucleotide (5'-GCAAGTTAACACG) containing no lesion, a thymine-thymine pyrimidine(6-4) pyrimidone photoproduct [TT(6-4)], or a cyclobutane pyrimidine dimer (TT-CPD) lesion (underlined) was inserted into the gapped-duplex pLL43/pKM71 creating an in frame *lacZ* 5' gene fragment.

Strains

All strains used in the present study are derivative of strain EMY74.7 (Johnson et al. 1998) (MATa his3-Δ1 leu2-3,112 trp1Δ ura3Δ met25Δ phr1Δ rad14Δ msh2Δ::hisG). Gene disruptions were achieved using PCR-mediated seamless gene deletion (Akada et al. 2006), URAblaster (Fonzi & Irwin 1993), or delitto perfetto (Storici & Resnick 2006) techniques. Strains used in the study are listed in Table S1.

All strains carry the plasmid pKM34 expressing cre recombinase, and the chromosomal integration cassette (lox66-3'lacZ-MET25) containing the 3' end of *lacZ* gene cloned from *Escherichia coli* MG1655 strain in fusion with the lox66 site. Integration cassettes were placed in two alternative locations within the yeast genome: near ARS306 or ARS606, using MET25 as selection marker.

Yeast Transformation

Plasmids carrying lesions were introduced into yeast cells by electroporation. Yeast cells were grown overnight to stationary phase in SC-TRP medium with 10 μg/ml doxycycline. The overnight culture was inoculated into 100 ml of yeast extract/peptone/dextrose (YPD) to reach OD_{600}=0.3. The cells were grown until OD_{600}=1.6 and harvested by centrifugation. The pellet was washed twice with 50 ml of cold water and once with 50 ml of cold electroporation

buffer (1 M sorbitol/1 mM $CaCl_2$). Cells were then incubated for 30 min at 30°C in conditioning buffer (0.1 M LiAc/10 mM DTT), collected by centrifugation, washed one more time with 50 ml of electroporation buffer, and then resuspended in 100–200 µl of the same buffer to reach 1 ml volume.

100 ng of plasmid DNA and 12 µg of carrier DNA was added to 400 ul of cell suspension and electroporated at 2.5 kV/25 mF/ 400 Ω in a BioRad GenePulser using 0.2 cm electrode gap cuvette. Typical electroporation time constant ranged from 3.0 to 4.5 ms. After electroporation, cells were suspended in 6 ml of 1:1 mix of 1 M sorbitol: YPDplus media (Zymo Research) and incubated at 30°C for 45 min. Finally, the cells were collected, resuspended in 5 ml 1M sorbitol, and plated on selective media using the automatic serial diluter and plater EasySpiral Dilute (Interscience).

Part of the cells were plated on SC-HIS to measure the transformation efficiency of plasmid pRS413 (internal transformation standard), and the rest was plated on SC-LEU (with 80 mg/ml X-gal and 10 µg/ml doxycycline) to select for integrants and visualize TLS and DA events as sectored blue-white and white colonies, respectively. Colonies were counted using the Scan 1200 automatic colony counter (Interscience). Lesion tolerance rates were calculated as the relative integration efficiencies of damaged vs non-damaged vectors normalized by the transformation efficiency of pRS413 plasmid in the same experiment. DA events are estimated by subtracting TLS events from the total lesion tolerance events.

The integration efficiency is about $1.5x10^5$ clones per picomole of non-damaged vector for a wild-type (WT) strain.

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Figure 1

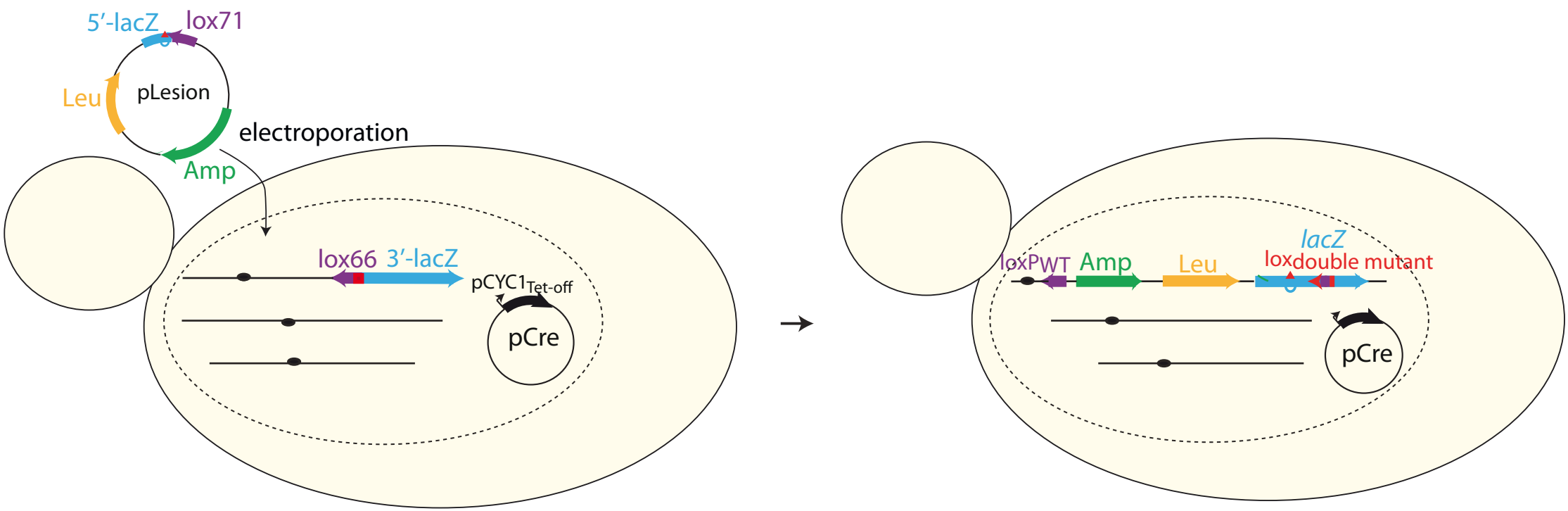
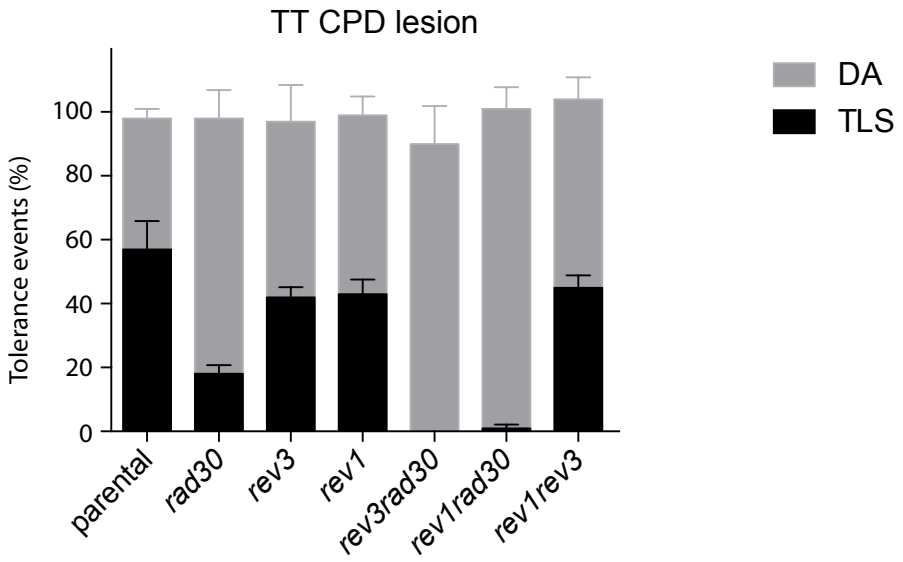

5′-lacZ
lox71
Leu
pLesion
Amp
electroporation
lox66
3′-lacZ
pCYC1Tet-off
pCre
loxPWT
Amp
Leu
lacZ
loxdouble mutant
pCre

Fig. 1: Introduction of a single lesion into a defined location in the yeast genome

A non-replicative plasmid containing a single lesion is integrated into a yeast chromosome using Cre/lox site-specific recombination. The integrative vector carrying a selection maker (LEU2) and the 5'-end of the *lacZ* reporter gene containing a single lesion is introduced into a specific locus of the chromosome with the 3'-end of the *lacZ*. The precise integration of the plasmid DNA into the chromosome restores a functional *lacZ* gene, allowing the phenotypical detection of TLS and DA events (as blue and white colonies on X-gal indicator media).

Figure 2


TT CPD lesion
Tolerance events (%)
0
20
40
60
80
100
parental
rad30
rev3
rev1
rev3rad30
rev1rad30
rev1rev3
DA
TLS

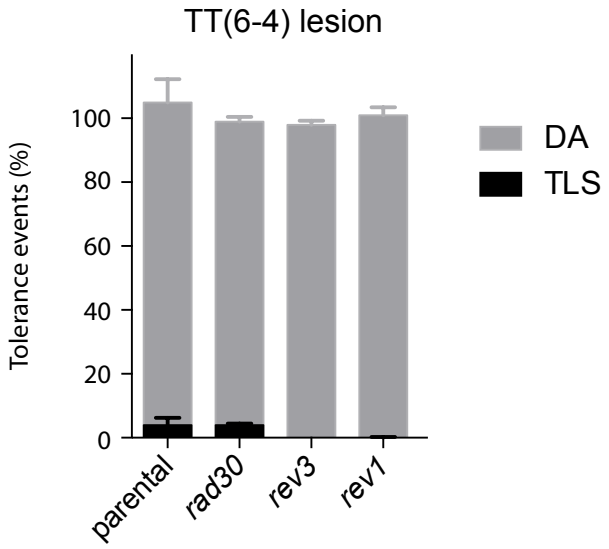

TT(6-4) lesion
Tolerance events (%)
0
20
40
60
80
100
parental
rad30
rev3
rev1
DA
TLS

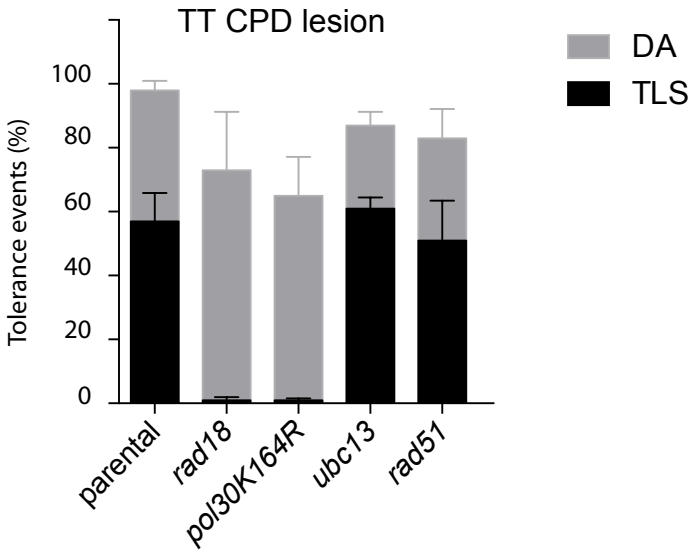
Fig. 2: Partitioning of DDT pathways in a strain deficient in TLS polymerases

The graph represents the partition of DDT pathways for two UV lesions, in strains deficient in TLS polymerases (Rev1, pol η/*rad30*, pol ζ/*rev3*). Tolerance events represent the percentage of cells able to survive in presence of the integrated lesion compared to the lesion-free control.

A. In a parental strain over 50% of events observed across a CPD lesion are TLS eventsDNA polymerase η is responsible for majority of CPD lesion bypass. However, in its absence TLS bypass of this lesion is still possible through the combined action of polymerase ζ and Rev1. In the absence of pol η removal of either Rev1 or pol ζ completely abolishes TLS.

B. For the TT(6-4) lesion, DA is the major tolerance pathway. Majority of TLS events through TT(6-4) lesion depend on pol ζ and Rev1, while pol η rarely contributes to the bypass of this lesion.

Figure 3


TT CPD lesion
DA
TLS
Tolerance events (%)
100
80
60
40
20
0
parental
rad18
pol30K164R
ubc13
rad51

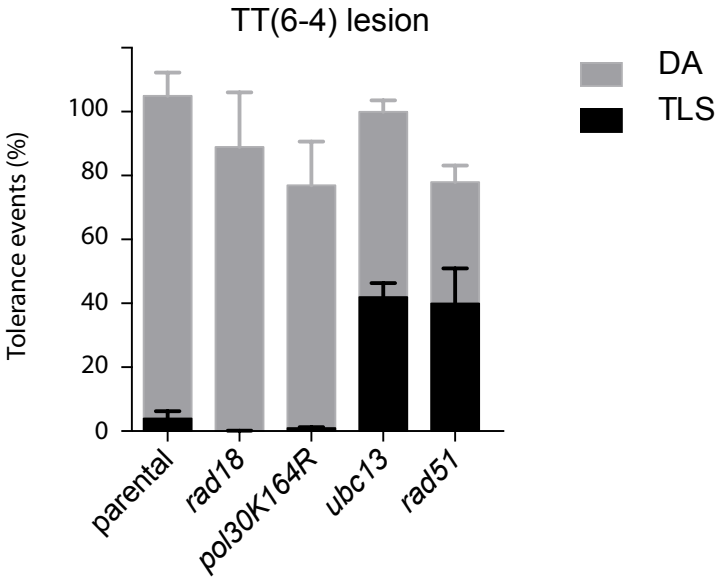

TT(6-4) lesion
DA
TLS
Tolerance events (%)
100
80
60
40
20
0
parental
rad18
pol30K164R
ubc13
rad51

Fig. 3: Partitioning of DDT pathways in the absence of PCNA ubiquitylation

Bypass of TT-CPD (A) and TT(6–4) (B) lesion in strains deficient in PCNA ubiquitylation (*rad18Δ* or *pol30-K164R*) or polyubiquitylation (*ubc13Δ*). Tolerance events represent the percentage of cells able to survive in presence of the integrated lesion compared to the lesion-free control.

In the absence of PCNA ubiquitiylation (*rad18Δ, pol30 K164R*) we observed a decrease in TLS. Remaining low level of TLS is probably due to polymerase interactions with PCNA ring not involving ubiquitin moiety. In the absence of PCNA polyubiquitylation (*ubc13Δ*), a small increase of the TLS bypass of the CPD lesion is observed, while Pol ζ-mediated bypass of the TT(6-4) lesion increased more than 10 fold. The absence of recombinase Rad51 results in a similar increase in TLS.